AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

Claim 1 (currently amended): A recrystallization inhibition method for determining at least one of the following: ~~the~~ presence, relative concentration defined by the concentration that is sufficient to exceed threshold of assay to known amounts of antifreeze protein, and ~~or~~ activity of thermal hysteresis proteins in a proteinaceous composition comprising:

providing a test solution comprising said proteinaceous composition in a solvent ~~to form a test solution~~;

providing at least one control solution;

flash freezing said test solution and said control solution to obtain a frozen test solution and frozen control solution;

raising the temperature of both said ~~the~~ frozen test solution and said frozen control solution to an appropriate annealing temperature that allows for a partial melt, while limiting heterogeneity in ice grain sizes within said test solution;

maintaining both said frozen test solution and said frozen control solution at the annealing temperature for a length of time sufficient to allow for ice recrystallization within said test solution;

monitoring ~~the~~ changes, by means of imaging, in ice crystal grain size ~~changes~~ in said test solution and said control solution over time; and

quantitative and statistical analysis of said imaging determining said presence, said relative concentration, and said activity of ~~functional~~ thermal hysteresis proteins of said test solution, while reducing the effect of non-antifreeze specific proteins, ~~in said~~

~~solution given the retention~~ based upon measurement of said ice crystal size and computation of a recrystallization inhibition (RI) factor relative to said ~~of significantly smaller ice crystal grain sizes relative to at least one~~ control solution.

Claim 2 (currently amended): The recrystallization inhibition method as defined in claim 1, wherein said solvent is selected from at least one of the group consisting of water, saline, and phosphate buffered saline (PBS)~~, or other isoosmotic inorganic or organic solutions~~.

Claim 3 (currently amended): The recrystallization inhibition method as defined in claim 1, wherein ~~two or more control solutions are used; where one control is~~ said control solution comprises one of the following: a solvent and ~~the other is~~ a ~~control~~ solution for non-specific recrystallization inhibition effects.

Claim 4 (cancelled)

Claim 5 (currently amended): The recrystallization inhibition method as defined in claim 1, wherein said proteinaceous composition is selected from the group consisting of: purified Tm ~~12.86. or~~ 12.86 ~~or~~ and purified Tm 12.84.

Claim 6 (original): The recrystallization inhibition method as defined in claim 1, wherein said proteinaceous composition is selected from the group consisting of antifreeze polypeptides, antifreeze glycopeptides, recombinant antifreeze polypeptides, recombinant antifreeze glycopeptides, synthetic antifreeze polypeptides analogs, synthetic antifreeze glycopeptide analogs, cell culture products, activators, recombinant bacterial products, recombinant products, uncharacterized plant products and transgenic plant products.

Claim 7 (cancelled)

Claim 8 (currently amended): The recrystallization inhibition method as defined in ~~claim 4~~ claim 1 wherein said proteinaceous composition ~~includes~~ comprises Tm 12.86 present in a concentration from about ~~between~~ 0.5 ug/ml to about 25 ug/ml.

Claim 9 (currently amended): The recrystallization inhibition method as defined in claim 1 ~~claim 2~~, wherein ~~the protein content in~~ said proteinaceous composition has a total protein content ~~is~~ less than or equal to 1 mg/ml in saline and phosphate buffered saline (PBS) ~~PBS~~; and less than or equal to 0.005 mg/ml in water.

Claim 10 (cancelled)

Claim 11 (currently amended): The recrystallization inhibition method as defined in ~~claim 10~~ claim 1, wherein ~~said~~ conditions to eliminate non-thermal hysteresis protein induced recrystalization conditions comprise ~~in~~ saline ~~are~~ at ~~6 (C~~ -6°C for 30 min with a total protein content less than or equal to 1 mg/ml; or in water at ~~2 (C~~ -2°C for 2 hours with a total protein content less than or equal to 0.005 mg/ml.

Claim 12 (currently amended): The recrystallization inhibition method as defined in claim ~~10~~ 11, under conditions ~~to~~ that avoid hyperosmotic solutions.

Claim 13 (currently amended): The recrystallization inhibition method as defined in claim 1, wherein ~~monitoring of~~ changes to said ice crystal grain size ~~changes~~ over time is monitored by a method chosen from: photomicroscopy, digital or video imaging.

Claim 14 (original): The recrystallization inhibition method as defined in claim 1, wherein quantitative data is collected by measurement of the mean largest ice grain size for both said test and control solutions to provide a basis for numerical assessment of the extent of recrystallization inhibition occurring.

Claim 15 (currently amended): The recrystallization inhibition method as defined in claim 14, wherein composite mean largest grain size (mlgs) are obtained for said test solution and said control solution; which are then statistically compared.

Claim 16 (original): The recrystallization inhibition method as defined in claim 1, wherein quantitative data collection is collected by assessment using a densitometer of light transmitted through a low magnification full view photographic negative of frozen sample wafer; absorbance peaks for said test solution is evaluated for maximum amplitude and statistically compared with said control solution.

Claim 17 (currently amended): The recrystallization inhibition method as defined in claim 1, wherein a dilution profile of said test solution is obtained over a wide dilution range until mean largest grain size (mlgs), or another quantifiably assessed response variable, are no longer significantly different from ~~the~~ at least one of: saline/phosphate buffered saline (PBS)~~/ and/or~~ , and non-temperature hysteresis proteins (THP) containing proteinaceous control solutions.

Claim 18 (currently amended) The recrystallization inhibition method as defined in claim 17, wherein composite mean largest grain size ~~mlgs~~, or absorbance peak area (light scattering), or computer generated units (digital/video imaging)~~,~~ are calculated for said test solution and plotted as a function of the logarithm of sample concentration, with replicate dilution series tested, and compared to control solution baseline.

Claim 19 (currently amended): The recrystallization inhibition method as defined in claim 17, wherein linear regression analyses is used to approximate the linear portion of the dilution profile, with application of a transforming function [arcsine[(mlgs)0.5] versus log(dilution)] to mean largest grain size ~~mlgs~~ to limit inherent curvature of dilution plots caused by the "leveling off" of ~~mlgs~~ mean largest grain size values for both very dilute and very concentrated thermal hysteresis protein samples.

Claim 20 (currently amended): The recrystallization inhibition method as defined in claim 1, wherein linear regression analyses provides the basis for development of a numerical factor (Relative Inhibition ~~RI~~ factor) describing the activity of the test solution with respect to recrystallization inhibition capability.

Claim 21 (original): The recrystallization inhibition method as defined in claim 20, wherein the RI factor is equal to the absolute value of the logarithm of the minimum test solution dilution required to eliminate recrystallization inhibition activity.

Claim 22 (original): The recrystallization inhibition method as defined in claim 21, wherein the RI factor is a measure of test solution recrystallization inhibition strength, according to the assessed exponential factor required for sufficient dilution of test solution to lose recrystallization inhibition activity, and providing a relative assessment of functional thermal hysteresis concentration within said test solution.

Claim 23 (currently amended): The recrystallization inhibition method as defined in claim 21, wherein the RI factor provides a relative assessment of functional thermal hysteresis protein concentration, and comparisons of various test solutions concentrations given translational shifts along the X axis showing log(dilution).

Claim 24 (original): The recrystallization inhibition method as defined in claim 19, wherein the regression line slope and Y-intercept reflect the recrystallization inhibition potency of a given test solution, thermal hysteresis protein species, recombinant thermal hysteresis protein product, synthetic thermal hysteresis analogue, or the like.

Claim 25 (original): The recrystallization inhibition method as defined in claim 19, wherein slope comparisons and shifts along Y-intercept provide relative potency comparisons between test solutions, thermal hysteresis species and the like.

Claim 26 (currently amended): The recrystallization inhibition method as defined in claim 20, wherein RI activity of a test solution is expressed as equivalent to the RI activity of a ~~expected concentrations~~ predetermined concentration of Tm 12.86 producing an equivalent RI ~~profiles~~ profile ~~are deduced, and provide reference interpretations of the test solution(s)' functional activity(ies) to an antifreeze protein of known characterized parameters experimentally measured~~.

Claim 27 (currently amended): The recrystallization inhibition method as defined in claim 22, wherein activity and potency of said test solution may include a combination of: more than one type of thermal hysteresis protein, ~~and/or~~ thermal hysteresis protein plus activator solutions such as in test solution of hemolymph, or artificial solutions containing predetermined ~~known~~ amounts of purified thermal hysteresis protein with an activator supplement.

Claim 28 (currently amended): The recrystallization inhibition method as defined in claim 1, further comprising mathematical modeling of the recrystallization inhibition process with prediction of effects on slope and Y-intercept and log/log transformations for test solution mean largest grain size ~~mlgs~~ data and analysis.

Claim 29 (currently amended): The recrystallization inhibition method as defined in claim 1, wherein the relationship between RI factors and thermal hysteresis levels for functionally active test solutions are described by the equation: RI factor = 1.428 LOG(TH) + 3.703.

Claim 30 (currently amended): The recrystallization method as defined in claim 1, wherein a random sampling method is used for data collection generating mean largest grain size ~~mlgs~~ which significantly eliminates the ~~impact~~ effect of intrasample ice crystal grain heterogeneity at ~~high~~ an annealing temperature in a range of approximately 2 - 4°C below a melting point of said test sample, and with at least one of the following: saline ~~/~~ and Phosphate Buffered Saline ~~PBS~~ solvents.

Claim 31 (original): The recrystallization inhibition method as defined in claim 1, further used for concurrent multiple sample testing of solutions.

Claim 32 (original): The recrystallization inhibition method as defined in claim 31, wherein said multiple testing of solutions includes the "sandwich" method; and application via a 96 well plate device.

Claim 33 (withdrawn): A method for quantitatively assessing the extent of recrystallization occurring in frozen foods, and the impact of solution additives to inhibit or limit recrystallization according to the process as defined in claim 1.

Claim 34 (withdrawn): A method for quantitatively assessing and comparing the effectiveness of cryoprotective solutions on the extent of recrystallization occurring in cryopreserved cells, tissues, solutions and the like, according to the process as defined in claim 1.